

**AMENDMENTS TO THE CLAIMS**

1-44. (Cancelled)

45. (Currently Amended) A method comprising:

flowing a fluid sample over a surface of a microfluidic channel;

allowing a sample component to bind with a binding partner disposed on the surface of the microfluidic channel;

flowing in series in the microfluidic channel a predetermined sequence of fluid plugs including first, second and third fluids, wherein the first and second fluids are separated by the third fluid which is immiscible with both the first and second fluids; and

accumulating an opaque material on a portion of the surface of the microfluidic channel.

46. (Original) The method of claim 45 further comprising determining the opacity of the opaque material.

47. (Cancelled)

48. (Original) The method of claim 46 wherein determining comprises irradiating the opaque material with light and measuring light transmittance.

49-50. (Cancelled)

51. (Original) The method of claim 45 wherein the sample component is one of an antigen and an antibody and the binding partner is the other of the antigen and the antibody.

52. (Original) The method of claim 45 wherein the fluid is passed over a plurality of surfaces.

53. (Original) The method of claim 52 wherein each of the plurality of surfaces is associated with a different binding partner.

54-71. (Cancelled)

72. (Previously Presented) The method of claim 45 wherein the opaque material comprises a metal.

73. (Previously Presented) The method of claim 72 wherein the metal comprises silver.

74. (Previously Presented) The method of claim 72 wherein the opaque material is formed by electroless deposition.

75. (Previously Presented) The method of claim 74 wherein the opaque material is electrolessly deposited on a metal colloid.

76. (Previously Presented) The method of claim 75 wherein the metal colloid comprises gold.

77. (Previously Presented) The method of claim 75 wherein the metal colloid comprises a gold-conjugated antibody.

78. (Previously Presented) The method of claim 45 wherein the opaque material is deposited over a region having a dimension of at least 10 microns.

79. (Previously Presented) The method of claim 45 wherein the opaque material is formed by flowing a metal solution.

80. (Previously Presented) The method of claim 79 wherein the metal solution comprises a silver salt.

81. (Previously Presented) The method of claim 72 further comprising measuring the conductivity of the accumulated metal.
82. (Previously Presented) The method of claim 46 wherein determining comprises irradiating the opaque material with light and measuring light reflectance.
83. (Previously Presented) The method of claim 45 wherein the sample comprises whole blood.
84. (Previously Presented) The method of claim 45 wherein the microfluidic channel comprises at least one cross-sectional dimension of less than 100 microns.
85. (Previously Presented) The method of claim 45 wherein the sample comprises urine.
86. (New) The method of claim 45, wherein the binding partner is disposed across a width of the microfluidic channel.
87. (New) The method of claim 45, wherein the opaque material forms a layer that extends across a width of the microfluidic channel.
88. (New) The method of claim 45, wherein the opaque material has a horizontal dimension of greater than 100 microns.
89. (New) The method of claim 48, wherein the light is pulse modulated.
90. (New) The method of claim 45, wherein the opaque material has a thickness of greater than 10 nanometers.

91. (New) The method of claim 45, wherein the binding and accumulating steps are performed while a fluid is flowing continuously in the microfluidic channel.
92. (New) The method of claim 45, further comprising quantitatively determining the opacity of the opaque material.
93. (New) The method of claim 45, wherein the first and second fluids are liquids and the third fluid is air.
94. (New) The method of claim 45, wherein the first and/or second fluids is a rinse solution.
95. (New) The method of claim 45, wherein the first fluid is a rinse solution and the second fluid is a metal solution.
96. (New) The method of claim 45, comprising introducing the first, second and third fluids into the microfluidic channel from a vessel containing the fluids in the predetermined sequence.
97. (New) The method of claim 96, wherein the vessel is sealed prior to use.
98. (New) The method of claim 97, wherein the first, second and third fluids are stored for greater than 1 month in the vessel prior to use.
99. (New) The method of claim 96, wherein the first, second and third fluids are introduced into the microfluidic channel by applying a vacuum.
100. (New) The method of claim 45, comprising exposing the opaque material to light of a first wavelength and detecting transmission of light of the first wavelength.

101. (New) The method of claim 45, comprising flowing in series over the binding partner disposed on the surface of the microfluidic channel the predetermined sequence of fluid plugs including the first, second and third fluids.

102. (New) The method of claim 45, wherein the accumulation step is performed after the sample is flowed over a surface of the microfluidic channel.